PATENT
034827-3002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Qu, K. et al.
Title:	COMPOSITIONS AND METHODS FOR DETERMINING GENOTYPES
Appl. No.:	10/714,508
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Art Unit	1637
Conf. No. :	9832

REPLY BRIEF

Mail Stop Appeal Brief - Patents
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Sir:

In response to the Examiner's Answer mailed September 19, 2007, Appellant submits this Reply Brief regarding the Final Rejection of claims 2 and 40-72. If any fee due is absent or incorrect, please charge or credit our Deposit Account No. 19-0741 for the appropriate amount. .

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Status of Claims

Claims 1 and 3-39 have been cancelled.

Claims 2 and 40-72 are pending in the application.

Claims 2 and 40-72 are the subject of this appeal.

Grounds for Rejection to be Reviewed on Appeal

1. Claims 2, 40-48, 50 and 67-72 stand finally rejected under 35 U.S.C. § 103(a), as allegedly being obvious over Lindpaintner et al. (N. Engl. J. Med., 332: 706-711, 1995; "Lindpaintner") in view of Lin et al. (Clin. Biochem., 34: 661-666, 2001; "Lin").

2. Claims 49 and 51-61 stand finally rejected under 35 U.S.C. § 103(a), as allegedly being obvious over Lindpaintner in view of Lin, Soubrier et al. (U.S. Patent 5,736,323; "Soubrier"), and Buck et al. (Biotechniques, 27: 528-536; 1999; "Buck").

3. Claims 62-65 stand finally rejected under 35 U.S.C. § 103(a), as allegedly being obvious over Lindpaintner in view of Lin and van Bockxmeer et al. (Circulation, 92: 2066-2071, 1995; "van Bockxmeer").

4. Claim 66 stands finally rejected under 35 U.S.C. § 103(a), as allegedly being obvious over Lindpaintner in view of Lin, van Bockxmeer, Soubrier, and Buck.

5. Claims 2, 40-48, 50 and 67-70 stand finally rejected under 35 U.S.C. § 103(a), as allegedly being obvious over Teranishi et al. (J. Hypertens., 17: 351-356, 1999); "Teranishi") in view of Lin.

Argument

1. Appellants' Claimed Invention.

The human ACE gene is known to have two allelic variants at Intron 16 based on the presence or absence of a 287 base pair non-coding fragment.[1] The allele which contains the non-coding fragment is referred to as the "I-allele", and the allele with the deletion (i.e., the deletion variant) is referred to as the "D-allele". These two alleles give rise to three possible genotypes: I/I, I/D, and D/D.

Appellants' claimed invention is a method for determining ACE genotype, or the genotype of any allele known to have a deletion variant, using a single PCR amplification. The claimed method is based on the use of three nucleic acid primers which consist of a flanking primer pair and a single deletion fragment-specific primer.[2] The flanking primer pair is selected to amplify the entire genomic region of interest, whether or not the deletion fragment is present. Amplification from the flanking primer pair results in a single amplicon from either allele, but the D-allele will produce an amplicon that is 287 nucleotides shorter than the amplicon produced from the I-allele.[3]

The deletion fragment-specific primer is designed to form a primer pair with one of the flanking primers and will only hybridize with, and amplify the I allele. Appellants disclose an exemplary deletion fragment-specific primer which results in an amplicon from the I-allele which is a characteristically different length than either amplicon produced by the flanking primers. Obviously, no amplicon is produced from the D-allele using this primer.[4] This third primer enables the production of a second amplicon from the I-allele.

The genotype of an individual may be determined using this three-primer system by merely counting the number of amplicons produced. Amplification of the D/D genotype results in only one (short) amplicon produced from the flanking primers. Amplification of the I/I

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[1] Specification at ¶ 5.

[2] Id. at ¶¶ 10 and 14.

[3] Id. at Figure 1.

[4] Id. at ¶ 14.

genotype results in two amplicons: one from the flanking primers and a second from the deletion fragment-specific primer. Finally, amplification of the I/D genotype results in three amplicons: the one amplicon from the D-allele and the two amplicons from the I-allele. Thus, identification of an individual's genotype may be determined using a single PCR reaction and merely counting the number of amplification products.[5]

As is discussed in more detail below, the production of two amplicons from the I allele using the three primer system is a critical distinction between Appellant's claimed method and the prior art. It is also the basis for producing a unique number of amplicons from each ACE genotype.

2. The claimed invention is unobvious over all rejections based on Lin et al.

All pending claims stand rejected as obvious over Lin et al. (Clin. Biochem., 34: 661-666, 2001; "Lin") in combination with other prior art references. Lin provides a method for determining an individual's ACE genotype at intron 16 using a configuration of primers that is virtually identical to Appellant's. Both Appellant and Lin utilize a three-primer system consisting of a flanking primer pair (e.g., "ACE1" and "ACE3" of Lin) and a single primer (e.g., "ACE2" of Lin), specific for the deletion fragment, which is designed to form a primer pair with one of the members of the flanking primer pair. For convenience, Appellant adopts the primer terminology of Lin for the remainder of this Brief.

Appellant's claimed method determines the ACE genotype of an individual by producing one, two, or three amplicons, wherein a unique number of amplicons is produced for each genotype. This simple genotyping methodology is based on the production of two amplicons from the I-allele and a single amplicon from the D-allele. The I-allele amplicons are produced from the ACE1-ACE3 and ACE2-ACE3 primer pair combinations.

By contrast to Appellant's method that produces three amplicons, Lin's method produces only two. One amplicon is produced from the D-allele using the ACE1-ACE3 primer pair, and one amplicon is produced from the I-allele using the ACE2-ACE3 primer pair. Thus, the D/D

[5] Id. at ¶ 55 and Figure 1.

genotype results in one amplicon. The I/I genotype results in one amplicon. And, the I/D genotype results in two amplicons. Lin identifies the presence or absence of D-amplicon and the I-amplicon using a melting curve analysis and identifies the genotype accordingly.

It is Appellant's production of two amplicons from the I-allele that distinguishes the claimed method from that of Lin. Unlike Appellant, Lin never detects or contemplates the production of an ACE1-ACE3 amplicon from the I-allele. Figure 3B of Lin provides an electrophoretic separation of Lin's PCR products. This figure clearly shows the 84-bp amplicon from the D-allele ("D form") and the 65-bp amplicon from the I-allele ("I form"). Missing from Figure 3B is a second amplicon from the I-allele that corresponds to the PCR product from the ACE1-ACE3 primer pair. This missing amplicon is expected to be about 371-bp; 287-bp larger than the D-allele amplicon, representing the presence of the deletion fragment.

In sum, Lin provides an ACE genotyping method using a three-primer system virtually identical to Appellant's primer configuration. And yet, despite the similarity, Lin arrives at a completely different result—the production of one amplicon from the I-allele instead of two. The failure of Lin to produce the second (larger) I-allele amplicon, or even contemplate its usefulness, is a testament to the unobviousness of Appellant's claimed method. In essence, Lin comes right to the edge of making Appellant's claimed invention, but stops short. Lin does not recognize the required modification (i.e., production of the second I-allele amplicon) or its utility. There is no stronger argument for unobviousness than this.

It is folly by the Examiner to attempt to combine Lin with other prior art ACE genotyping methods in order to provide the missing third amplicon. First, in doing so, the Examiner has completely missed the significance of Lin as it relates to the appealed claims. Lin provides the primers necessary to perform Appellant's claimed method and yet fails to appreciate the possibility of producing two amplicons from the I-allele. This fundamental oversight alone renders Appellant's claimed invention unobvious because, how can a person of ordinary skill be expected to arrive at the claimed invention when Lin cannot?

Second, there is no motivation to produce a third amplicon (i.e., a second amplicon from the I-allele) in the Lin method. Lin provides a complete system for determining ACE genotype which is based on producing a single amplicon from each of the D- and I-alleles. The presence

or absence of these two amplicons is detected using a melting curve analysis. The introduction of a third amplicon into the method of Lin, as suggested by the Examiner, does not provide any additional information regarding the ACE genotype and has the possibility to confound the genotype determination. A third amplicon would make the melting curve analysis more complex and may interfere with the interpretation/identification of the two amplicons already present. Accordingly, a skilled artisan would not be motivated to modify the Lin method as suggested by the Examiner.

For the foregoing reasons, Appellant respectfully submits that all rejections are traversed because they are all based on a flawed analysis of Lin and its combination with the prior art. Appellant further submits that all rejections should be reversed and withdrawn.

Although the foregoing arguments apply equally to all grounds of rejection, Appellant addresses each rejection individually below.

3. New grounds of rejection and Appellant's response.

The Examiner's Answer is remarkable is several respects, not least of which is the assertion of substantial new rejections based on prior art not previously relied upon (Hiratsuka et al.). Although it is within Appellant's right to demand that prosecution be reopened, Appellant will address these rejections for the first time in this paper. It is Appellant's sincere desire to expedite prosecution and resolve all outstanding issues and rejections.

3.1 Claims 2, 40-48, 50, and 67-70

Claims 2, 40-48, 50, and 67-70 stand rejected as obvious over Lin et al. (Clin. Biochem., 34: 661-666, 2001; "Lin") in view of Hiratsuka et al. (Anal. Biochem. 289: 300-303, 2001; "Hiratsuka"). Appellant traverses this rejection.

Lin provides a real-time PCR-based method for determining the ACE genotype at intron 16 using a three-primer system consisting of a flanking primer pair (ACE1 and ACE3) and primer specific for the deletion fragment (i.e., hybridizes only to the I allele; ACE2). Lin reports

detecting a single 84-bp amplicon from the D-allele (ACE1-ACE3 primer pair) and a single 65-bp amplicon from the I-allele (ACE2-ACE3 primer pair).[6] Lin never produces or detects an I-allele amplicon produced from the ACE1-ACE3 primer pair which, theoretically, would be significantly longer than the other two (see, Figure 1).

After amplification, Lin determines the ACE genotype using a melting curve analysis. The 65-bp amplicon from the I-allele has a lower melting point than the 84-bp amplicon from the D-allele. Thus, homozygotes are identified by the presence of a single melting curve peak with a unique melting temperature based on the amplicon length (65-bp or 84-bp), and heterozygotes are identified by the presence of two melting curve peaks (65-pb and 84-bp) (see, for example, Figure 3A). Lin, therefore, determines the ACE genotype of an individual using no more than two amplicons and a genotype is assigned based on physical properties (i.e., melting temperature) of those amplicons.

In asserting this rejection, the Examiner mischaracterizes Lin. The Examiner alleges that Lin

> detect[s] a homozygous ACE genotype by the production of one or two amplification products (Figure 3, where homozygous ACE genotypes are detected by the production of one or two amplification products, see melting peaks for the I/I or D/D genotypes).[7]

This is clearly incorrect. As discussed above, Lin produces only two amplicons; one from the I-allele and one from the D-allele. Thus, either homozygote (i.e., I/I or D/D) is identified by the presence of only a single amplicon. The heterozygote is identified by the presence of two amplicons.

This misunderstanding is a critical point of distinction between Appellant's claimed method and that of Lin. Lin's implementation of the three-primer system produces a single amplicon from each of the I- and D-alleles of the ACE gene. Appellant's claimed method produces one amplicon from the D-allele and two amplicons from the I-allele. Production of the

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[6] Page 662, Section 2.4 notes that the primers were the same as described by Evans et al. Page 663, right column notes that Evans et al. reported fragments of 65 bp and 84 bp for the I- and D-alleles respectively. These fragment lengths are consistent with Figure 1.

[7] Examiner's Answer at p. 21, end of second paragraph.

second amplicon from the I-allele permits Appellant to identify each ACE genotype by the production of a unique number of amplicons. Specifically, one amplicon is produced from the D/D genotype, two amplicons are produced from the I/I genotype, and three amplicons are produced from the I/D genotype. Appellant's method eliminates the need for further analysis or differentiation among amplicons (e.g., by melting curve analysis as required by Lin) because a unique number of amplicons is produced for each of the three possible genotypes.

Recognizing on appeal that Lin does not produce or detect three different amplicons,[8] the Examiner attempts to supplement Lin with Hiratsuka. However, Hiratsuka, like Lin, provides an ACE genotyping method that produces only a single amplicon from each allele. Thus, Hiratsuka is an alternative genotyping methodology to Lin. There is no motivation to combine these methods and, even when combined, do not result in Appellant's claimed method.

Hiratsuka provides a simple two-primer genotyping method. The two primers correspond to the "ACE1" and "ACE3" flanking primers of Lin. Amplification of the ACE gene using these primers results in a 373-bp amplicon from the I-allele and an 85-bp amplicon from the D-allele.[9] The amplicons are distinguished based on a melting curve analysis in which the longer amplicon has a different melting profile than the shorter amplicon. A homozygous ACE genotype is assigned based on detecting one of the melting profiles, and the heterozygous genotype is assigned when a compound melting profile is observed.

Fundamentally, Hiratsuka provides the same protocol as Lin. Each method produces a single amplicon from each allele and distinguishes the allele-specific amplicons based on melting curve analysis. The difference between the two methods is merely the choice of primer strategy and primer positioning. Lin utilizes three primers and achieves two amplicons of similar, but distinguishable length, whereas Hiratsuka utilizes a flanking primer pair that results in two amplicons where the difference in length is equal to the size of the deleted sequence.

In a misguided attempt to combine the methods of Lin and Hiratsuka, the Examiner states that "while Lin teaches the three primers and does not detect the third amplicon, Hiratsuka

[8] Examiner's Answer at p. 23, ¶ 4.

teaches that the product of these flanking primers would be expected to detect a product when the insertion is present that is 387 based pairs in length."[10] The Examiner concludes, therefore, that the production of a third amplicon from the three-primer system of Lin is obvious.

The question here is not whether a third amplicon could have been produced by Lin from the ACE1-ACE3 primer pair (clearly it is possible), but rather, whether one would have been motivated to produce such an amplicon and whether it would be useful in Lin's method. Clearly, the answer to both questions is no. The addition of a third amplicon is unnecessary and may confound genotype determination by introducing a third melting peak without providing any additional information regarding the ACE genotype.

In sum, Lin and Hiratsuka teach variations on the same method, not complementary methods. As a result, the Examiner has failed to identify every element of Appellant's claimed invention in the cited prior art and has failed to identify a motivation to combine the prior art elements in a manner that results in the claimed invention. Specifically, the Examiner has failed to demonstrate a method for determining ACE genotype which produces a unique number of amplicons for each genotype because no cited method produces two amplicons from the I-allele. The proper combination of Lin and Hiratsuka may result in amplicons of different lengths, but would not result in a third amplicon (i.e., a second amplicon from the I-allele). Furthermore, there is no motivation to produce a second amplicon from the I-allele because it may confound identification of the characteristic I- and D-allele amplicons already produced, but would not provide any additional information. Thus, the Examiner has failed to make a *prima facie* case for obviousness. This rejection should be reversed and withdrawn.

3.2 Claims 49 and 51-61

Claims 49 and 51-61 stand newly rejected as obvious over Lin in view of Hiratsuka and in further view of Soubrier et al. (U.S. Patent 5,736,323; "Soubrier") and Buck et al. (Biotechniques, 27: 528-536, 1999; "Buck"). Appellant traverses this rejection.

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[9] Hiratsuka et al. p. 301, left column, last three lines.

[10] Examiner's Answer at p. 24, ¶ 2.

As an initial matter, claims 49 and 51-62 each depend directly or indirectly from claim 2. For the reasons discussed above, Appellant submits that claim 2 is unobvious over the basic combination of Lin and Hiratsuka. Soubrier and Buck are not alleged by the Examiner to address the deficiencies of this combination of references. Accordingly, these dependent claims are also unobvious and this rejection should be reversed and withdrawn.

Claims 49, 51-52, and 54-60 require that the three amplicons are approximately (or exactly) 123-bp, 157-bp, and 410-bp in length and/or are produced using specific primers. The Examiner alleges that Lin provides amplicons that are 65-bp and 84-bp in length, and Hiratsuka provides an amplicon that is 373-bp in length, which correspond to Appellant's claimed amplicons, respectively.[11] The Examiner further alleges that Soubrier teach the full-length sequence of intron 16 and that Buck teaches the equivalency of primers. The Examiner concludes that the production of amplicons having the specified lengths and/or produced using the specified primers is merely routine optimization of the Lin/Hiratsuka method based on the known sequence of intron 16, provided by Soubrier. The artisan has an expectation of success because Buck teaches the equivalency of primers.

Contrary to the Examiner's allegation, the cited prior art does not teach or suggest amplicons having the specified lengths. The allegation that the 65-bp and 84-bp amplicons of Lin are approximately 123-bp and 157-bp in length, respectively, is ridiculous and contrary to the definition of "approximately" provided in the Specification. Each of Appellant's claimed amplicons is about twice as long as those of Lin. Under any reasonable interpretation, these cannot be said to be "approximately" equal in length. Furthermore, Appellant defines "approximately" as encompassing lengths ± 10-20%.[12] Thus, the prior art amplicons are clearly outside the scope of claims 49, 51-52, and 54-60.

Appellant respectfully disagrees that Buck teaches the equivalency of amplification primers that are suitable for use in a multiplex amplification reaction, as required for the instant

[11] Examiner's Answer at p. 26, ¶ 1 and p. 27, ¶ 4.

[12] Specification at ¶ 30.

invention. As discussed in Appellant's Appeal Brief,[13] Buck teaches the equivalency of sequencing primers. These primers are used individually and must be sufficient to prime a primer extension reaction that is randomly terminated prior to completion of a full copy of the target DNA. Thus, a certain level of primer failure (i.e., early termination of extension) is acceptable. By contrast, a PCR reaction creates a full copy of the target DNA and the primers are used in pairs to define the boundaries of the target DNA. The Examiner has not demonstrated the equivalency between primers suitable for sequencing and those suitable for multiplex PCR.

This rejection, insofar as it applies to claims 49, 51-52, and 54-60, fails to provide amplicons of the size specified in the rejected claims and further fails to provide a reasonable expectation of modifying the genotyping methods of Lin and Hiratsuka to produce appropriately sized amplicons. The rejection of claims 49, 51-52, and 54-60 is traversed and should be withdrawn.

Claims 53 and 61 further define a specific amplicon arrangement for ACE genotype determination. The I/I genotype is identified by the presence of two amplicons (the "second and third fragments"). The D/D genotype is identified by the presence of a single, different amplicon ("the first fragment"). And, the I/D genotype is identified by the presence of all three of the above amplicons. The Examiner alleges that the combination of Lin and Hiratsuka teaches an embodiment of these claims.[14]

Appellant traverses this rejection because, as already demonstrated, neither Lin nor Hiratsuka teach or suggest an ACE genotyping method that produces three amplicons. Neither Lin nor Hiratsuka produce a second amplicon from the I-allele. Accordingly, the homozygous genotypes each are identified by the presence of a single amplicon, and the heterozygous genotype is identified by the presence of two amplicons. The Examiner cannot account for the three amplicons required in claims 53 and 61 using the methods of Lin and Hiratsuka. The rejection of claims 53 and 61 is traversed and should be withdrawn.

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[13] Appeal Brief at Section 4.2.

[14] Examiner's Answer at p. 26, ¶ 2 and paragraph spanning pp. 27 and 28.

3.3 Claims 62-65

Claims 62-65 stand newly rejected as obvious over Lin in view of Hiratsuka, in further view of van Bockxmeer et al. (Circulation, 92: 2066-2071, 1995; "Bockxmeer"). Appellant traverses this rejection.

The Examiner cites the combination of Lin and Hiratsuka as discussed above. The Examiner cites van Bockxmeer to demonstrate that the prior art recognized an association between an individual's ACE genotype and their likelihood of developing certain cardiovascular diseases. Appellant agrees with this characterization of van Bockxmeer but points out that it does not remedy the basic deficiency in the combination of Lin and Hiratsuka, as applied to claims 62-65. This rejection is traversed and should be withdrawn.

3.4 Claim 66

Claim 66 stands newly rejected as obvious over Lin in view of Hiratsuka, in further view of van Bockxmeer, Soubrier et al. (U.S. Patent 5,736,323; "Soubrier") and Buck. Appellant traverses this rejection.

Claim 66 depends from claim 62. As discussed above, the basic combination of Lin and Hiratsuka does not render obvious Appellant's claimed method of determining ACE genotype. Soubrier and Buck do not remedy the deficiency of the combination of Lin and Hiratsuka, nor do Soubrier and Buck teach Appellant's specific primers recited in claim 66. Soubrier merely provides the DNA sequence of ACE intron 16 and identifies the polymorphic fragment. Buck provides a general teaching that many/most sequencing (not PCR amplification) primers for a given DNA sequence may be successfully used in a sequencing reactions. Thus, there is no motivation to combine the teachings of Buck with those of Soubrier because they are concerned with separate types of polymerase reactions; Soubrier with an amplification reaction and Buck with a sequencing reaction.

Specifically absent from the teachings of Soubrier and Buck is a suggestion to use three primers in an amplification reaction in order to assess the presence or absence of a polymorphic insertion in such a manner that each genotype results in a distinct number of amplicons. Further,

neither Soubrier nor Buck specifically teach or suggest using Appellant's primers of SEQ ID NOs: 1-3.

Finally, van Bockxmeer merely correlates ACE genotypes with certain cardiovascular diseases. Nothing in van Bockxmeer addresses the specific deficiencies of the ACE genotyping methodology based on the combination of Lin and Hiratsuka. Accordingly, this rejection is traversed and should be withdrawn and such action is respectfully requested.

3.5 Claims 71-72

Claims 71-72 stand newly rejected as obvious over Lin in view of Hiratsuka. Appellant traverses this rejection.

Claims 71-72 depend from claim 67 which encompasses a method for determining a genotype of interest by amplifying sample DNA in a single reaction using a three-primer system consisting of a flanking primer pair and an internal primer capable of forming a primer pair with one of the flanking primers. The reaction produces either one or two amplicons from the homozygous genotypes and three amplicons from the heterozygous genotype. Claims 71-72 require that the three amplicons are approximately (or exactly) 123-bp, 157-bp, and 410-bp in length. The Examiner alleges that Lin provides amplicons that are 65-bp and 84-bp in length, and Hiratsuka provides an amplicon that is 373-bp in length, which correspond to Appellant's claimed amplicons, respectively.[15]

For the reasons discussed above, the combination of Lin and Hiratsuka do not render obvious Appellant's claimed method. Specifically, this combination of references does not provide a method which produces one, two, or three amplicons, depending upon the ACE genotype, as claimed by Appellant. Additionally, there is no motivation to combine the methods of Lin and Hiratsuka because they are essentially the same method rather than complementary methods. Furthermore, contrary to the Examiner's allegation, the cited prior art does not teach or suggest amplicons having the specified lengths. The allegation that the 65-bp and 84-bp amplicons of Lin are approximately 123-bp and 157-bp in length, respectively, is contrary to the

[15] Examiner's Answer at paragraph bridging pages 39 and 40.

definition of "approximately" provided in the Specification. Appellant defines "approximately" as encompassing lengths ± 10-20%.[16] Claim 72 requires that the amplicons are exactly 123, 157, and 410-bp in length. These specific lengths are not taught or suggested by Lin or Hiratsuka. Thus, the prior art amplicons are clearly outside the scope of claims 71-72. This rejection is traverse and should be withdrawn.

4. Existing Rejections and Appellant's response to Examiner's arguments.

4.1 Rejections over Lin and Lindpaintner

All pending claims stand rejected as obvious over the combination of Lin and Lindpaintner et al. (N. Engl. J. Med., 332: 706-711; 1995; "Lindpaintner") either alone or in combination with Soubrier, Buck, and/or van Bockxmeer.

As discussed above, Lin provides a real-time PCR-based method for determining ACE genotype using a three-primer method that produces two distinct amplicons: an 84-bp amplicon from the D-allele and an 65-bp amplicon from the I-allele. Using melting curve analysis, Lin is able to identify and distinguish these two amplicons and assign the appropriate ACE genotype.

Lindpaintner describes a nested-PCR method that requires two steps and four-primers to definitively determine all ACE genotypes. In the first step, genomic DNA is amplified using a flanking primer pair (hace3s and hace 3as) in order to amplify the entire sequence of interest. The result is a long and a short amplicon corresponding to the I- and the D-alleles, respectively.[17] This PCR product is initially screened to identify ACE genotype, wherein the presence of only the long amplicon definitely indicates the I/I genotype and presence of both amplicons definitely indicates the I/D genotype. Any sample having a putative D/D genotype is subjected to a second PCR reaction for confirmation.

Lindpaintner does not definitely identify the D/D genotype from the result of the first PCR amplification because the shorter D-allele amplicon is preferentially amplified relative to the I-allele amplicon. Thus, samples lacking the I-allele amplicon (i.e., putative D/D after the

[16] Specification at ¶ 30.

[17] Lindpaintner at p. 707, right column, second paragraph; amplicons of 319-bp and 597-bp result from the D-allele and the I-allele, respectively.

first PCR amplification) must be further tested to confirm that its absence is truly indicative of a D/D genotype and not an artifact of differential amplification. Confirmation is done using a second PCR with a different primer pair (hace5a and hace5c) in which one member binds specifically to the insertion sequence.[18] The I-allele, which contains the insertion sequence, gives rise to an amplification product; whereas, the D-allele, which lacks the fragment (i.e., lacks one primer binding site), does not yield an amplification product. Based on this secondary analysis, the samples lacking the I-amplicon from the first PCR are categorized as the I/D genotype (presence of amplicon) or the D/D genotype (absence of amplicon).

Genotype identification using the Lindpaintner method is as follows: The I/I genotype is identified by one single amplicon in the first PCR (the second PCR being unnecessary). The D/D genotype is identified by one single amplicon in the first PCR and the absence of an amplicon in the second PCR. The I/D genotype is identified either by the presence of two amplicons in the first PCR (the second PCR being unnecessary) or by the presence of the D-amplicon in the first PCR and the I-amplicon in the second PCR. Thus, Lindpaintner identifies every genotype based on the presence of either one or two amplicons. Lindpaintner never requires the production of three amplicons to identify the I/D genotype, as required in Appellant's rejected claims.

In an attempt to demonstrate that the prior art suggests utilizing three amplicons to identify the I/D genotype, the Examiner points out that Lindpaintner produces two amplicons from the I-allele. While this is technically a correct statement, it is a misrepresentation of the prior art method because Lindpaintner never uses both I-allele amplicons together. According to the method of Lindpaintner, one I-allele amplicon may be produced in the first PCR, and the second I-allele amplicon may be produced in the second PCR. However, production of the I-allele amplicon in the first PCR renders the second PCR unnecessary because a definitive identification of either the I/I or I/D genotype can be made. It is only in the absence of an I-allele amplicon in the first PCR that a second PCR is performed. Thus, contrary to the Examiner's implication, there is no instance in which it is necessary for Lindpaintner to produce two I-allele amplicons in order to determine an ACE genotype.

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[18] Lindpaintner at p. 707, right column, third paragraph.

The Examiner has also failed to address the lack of motivation to combine the methods of Lin and Lindpaintner. Lin provides a single-step real-time PCR method for determining ACE genotype. Lin validates this method against a "conventional PCR" ACE genotyping method which is substantially identical to the method of Lindpaintner. Specifically, Lin performs parallel genotype determinations using the 2-step nested-PCR method described in Section 2.3.[19] This "conventional PCR" method uses the same primer strategy as that used by Lindpaintner, and achieves the same results. It is clear that the Lin method is offered as an alternative to the traditional two-step nested PCR method of Lindpaintner, not as an improvement or a compatible method. Furthermore, as discussed above, the skilled artisan is not motivated to produce a third amplicon from the Lin method which has the possibility to confound the melting curve analysis without providing additional information regarding ACE genotype. Thus, a skilled artisan would not combine the real-time PCR method of Lin with the nested PCR method of Lindpaintner; he would use the Lin method instead of the Lindpaintner method.

In sum, both Lin and Lindpaintner fails to teach a method for the simultaneous production of two amplicons from the I-allele. This prevents either method (or the combination of methods) from producing a unique number of amplicons from each ACE genotype. Furthermore, there is no motivation to combine the teachings of Lindpaintner and Lin. The Lin method was specifically developed as an alternative to the traditional (Lindpaintner) method of ACE genotyping which requires two separate PCR amplifications.

Appellant respectfully submits that all rejected claims are not obvious over the combination of Lin and Lindpaintner and requests that this rejection be reversed and withdrawn.

4.2 Rejections over Lin and Lindpaintner in view of Soubrier and Buck

Soubrier provides the sequence of intron 16 (1856 nucleotides) and specifically identifies the 287 nucleotide polymorphic fragment. Soubrier also suggests that primer pairs may be used to amplify all or a portion of intron 16 for the purpose of determining whether or not the polymorphic fragment is present. However, Soubrier does not teach or suggest the specific primers of the rejected claims.

[19] Lin et al. at p. 662, "2.3. Genotyping of ACE gene I/D allele by conventional PCR".

Buck demonstrates the equivalence of sequencing primers. Appellant respectfully disagrees that Buck teaches the equivalency of amplification primers that are suitable for use in a multiplex amplification reaction, as required for the instant invention. As discussed in Appellant's Appeal Brief,[20] Buck teaches the equivalency of sequencing primers. These primers are used individually and must be sufficient to prime a primer extension reaction that is randomly terminated prior to completion of a full copy of the target DNA. Thus, a certain level of primer failure (i.e., early termination of extension) is acceptable. By contrast, a PCR reaction creates a full copy of the target DNA and the primers are used in pairs to define the boundaries of the target DNA. The Examiner has not demonstrated the equivalency between primers suitable for sequencing and those suitable for multiplex PCR.

Taken together, mere knowledge of the sequence of intron 16, combined with a general teaching related to sequencing primers does not render obvious the specific primers of the rejection claims, let alone Appellant's claimed method for determining ACE genotype.

4.3 Rejections over Teranishi and Lin

Claims 2, 40-48, 50, and 60-70 stand rejected as obvious over the combination of Teranishi et al. (J. Hypertension, 17: 351-356, 1999; "Teranishi") and Lin. Appellants respectfully request that the rejection be reversed and withdrawn.

Lin provides a real-time PCR-based method for determining ACE genotype using a three-primer method that consists of a flanking primer pair (ACE1 and ACE3) and an insertion fragment-specific primer (ACE2). Lin produces two distinct amplicons: an 84-bp amplicon from the D-allele and an 65-bp amplicon from the I-allele. Using melting curve analysis, Lin is able to identify and distinguish these two amplicons and assign the appropriate ACE genotype.

The method of Lin differs from Appellant's claimed method in that Lin does not produce a distinct number of amplicons for each ACE genotype. Specifically, Lin does not teach or suggest producing two amplicons from the I-allele.

Teranishi does not remedy the deficiency of Lin and is only an incremental change from the method of Lindpaintner. Teranishi, like Lindpaintner, performs a two-step nested PCR

[20] Appeal Brief at Section 4.2.

method in order to determine an ACE genotype. The sole difference between the methods of Lindpaintner and Teranishi is that Teranishi reuses one of the flanking primers of the first PCR reaction in the second PCR reaction.[21] However, this does not change the fundamental strategy of a two-step nested PCR. Thus, Teranishi's output is substantially identical to that of Lindpaintner. Specifically, Teranishi never detects more than two amplicons in order to identify any genotype and Teranishi requires two PCR amplifications to identify all genotypes. Nowhere does Teranishi does not suggest combining the three primers into a single PCR reaction.

The combination of Lin and Teranishi, like the combination of Lin and Lindpaintner, fails to teach every limitation of Appellants' claimed invention because neither reference teaches a method for the simultaneous production of two amplicons from the I-allele or a method for determining ACE genotype by producing a different number of amplicons from each. Furthermore, there is no motivation to combine the teachings of Teranishi and Lin because the Lin methodology is proposed as an alternative to the "conventional" two-step nested PCR method of Teranishi. Applicants respectfully submit that claims 2, 40-48, 50 and 67-70 are not obvious over Teranishi in view of Lin and request that this rejection be reversed and withdrawn.

Conclusion

For the reasons discussed above, Appellants respectfully submit that claims 2 and 40-72 are in condition for allowance, and respectfully request that the rejections be reversed and withdrawn, and that the claims be allowed to issue.

[21] Teranishi at p. 352, left column, ¶ 3.

Respectfully submitted,

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